

BEST AVAILABLE COPY

Figure 1

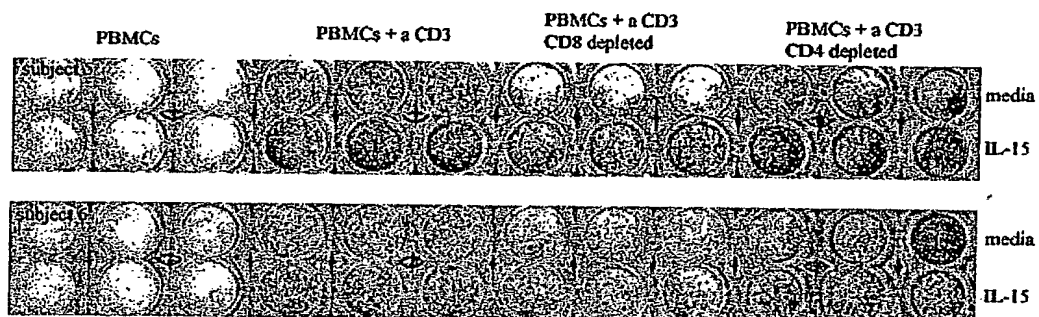


Figure 2

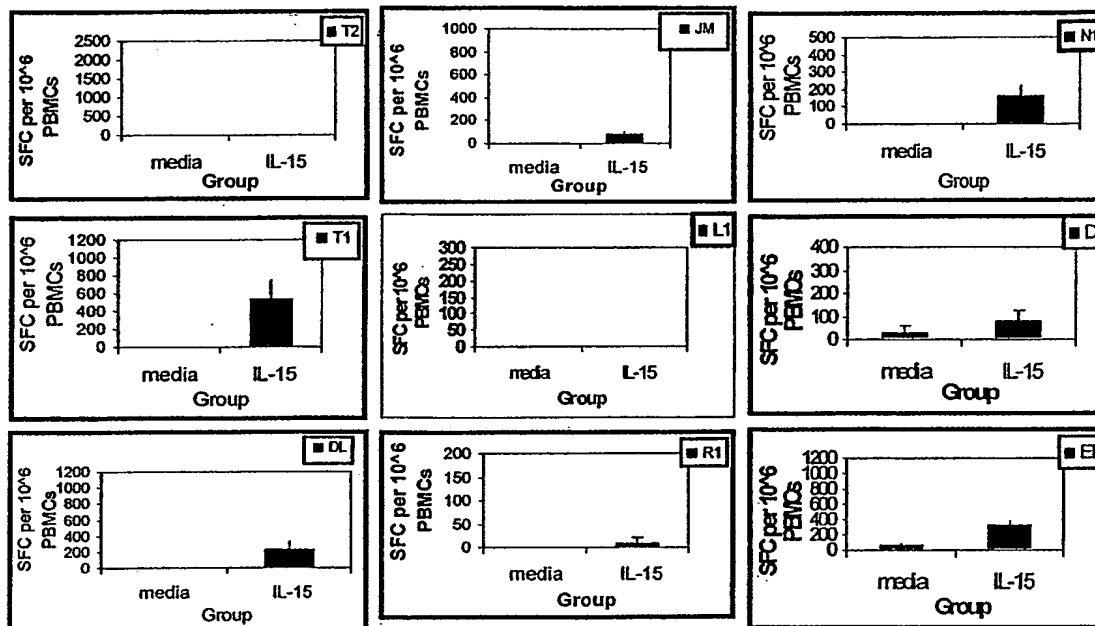


FIGURE 3A

BEST AVAILABLE COPY

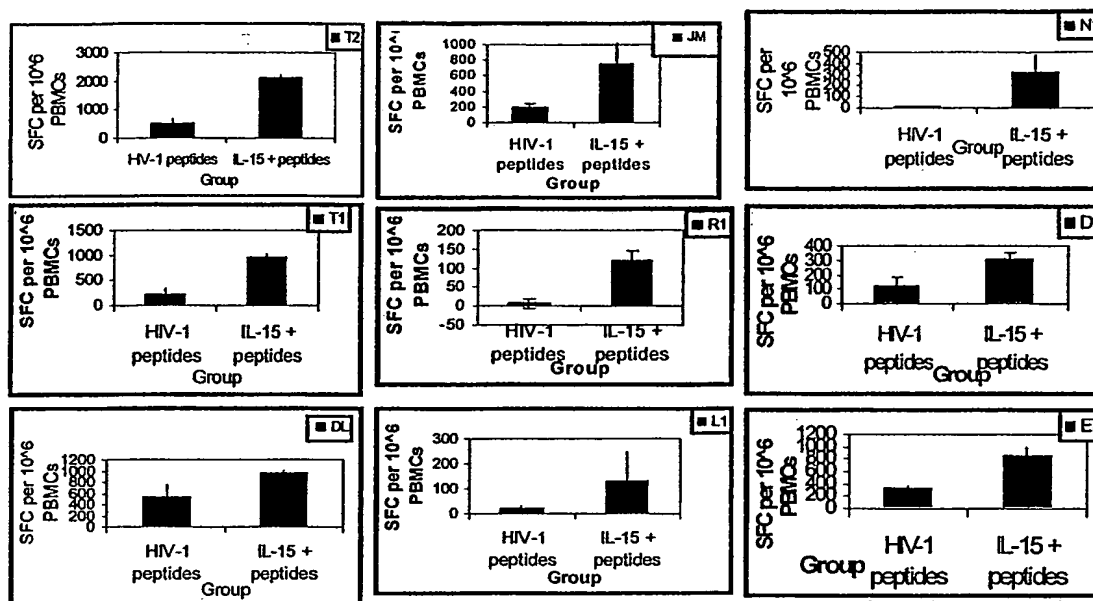


FIGURE 3B

BEST AVAILABLE COPY

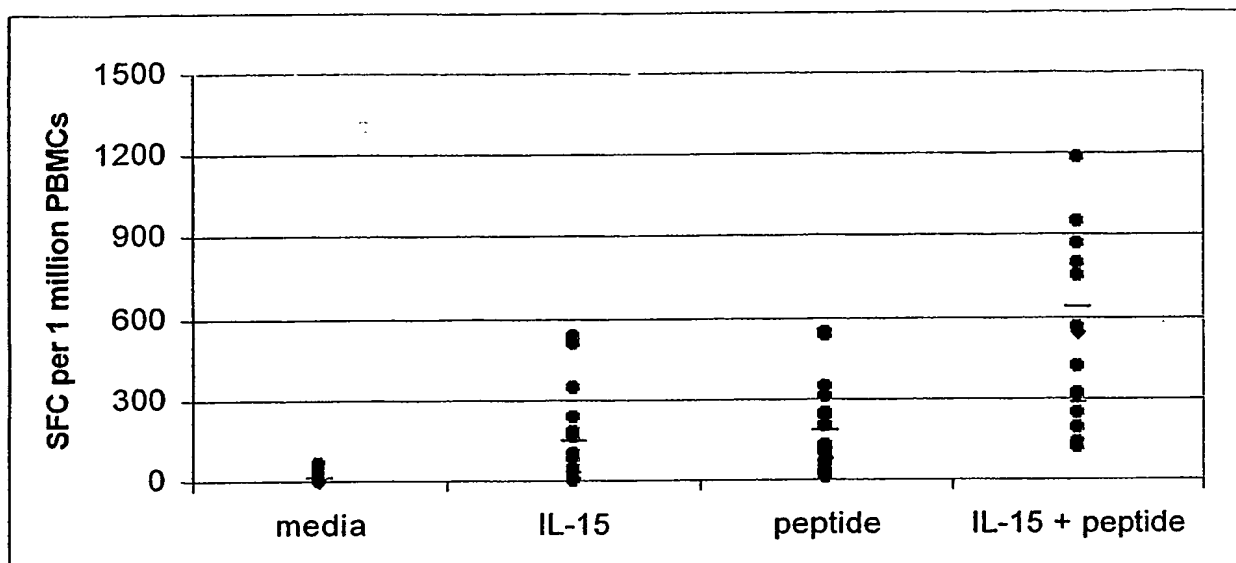


Figure 3C

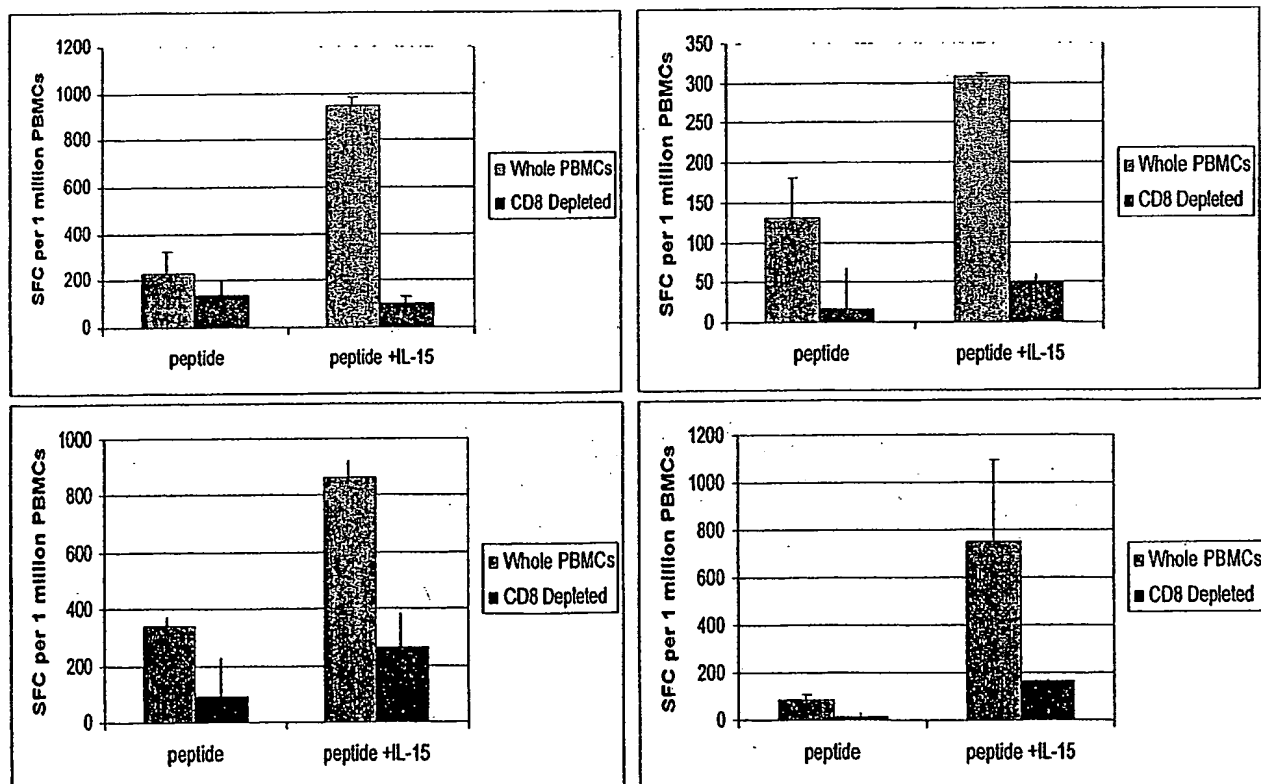


Figure 3D

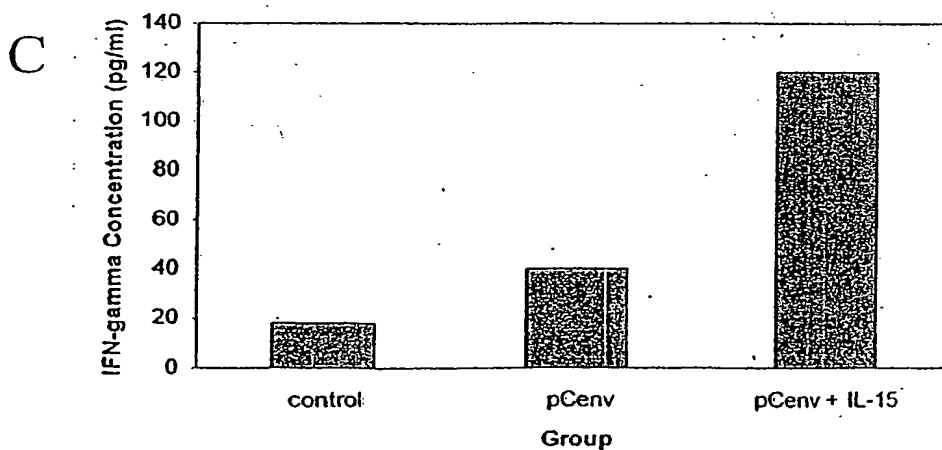
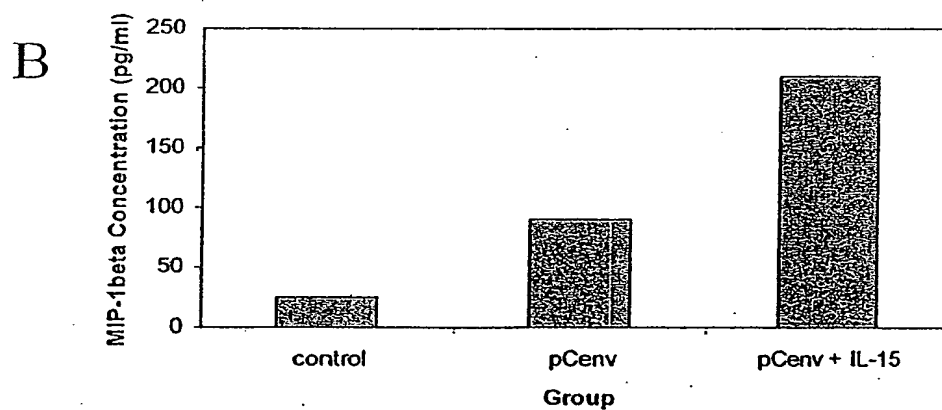
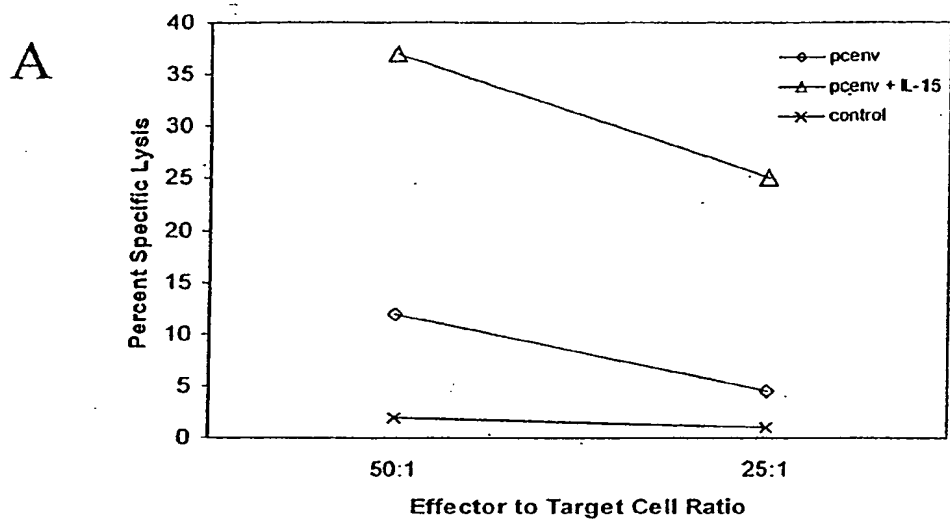


Figure 4

BEST AVAILABLE COPY

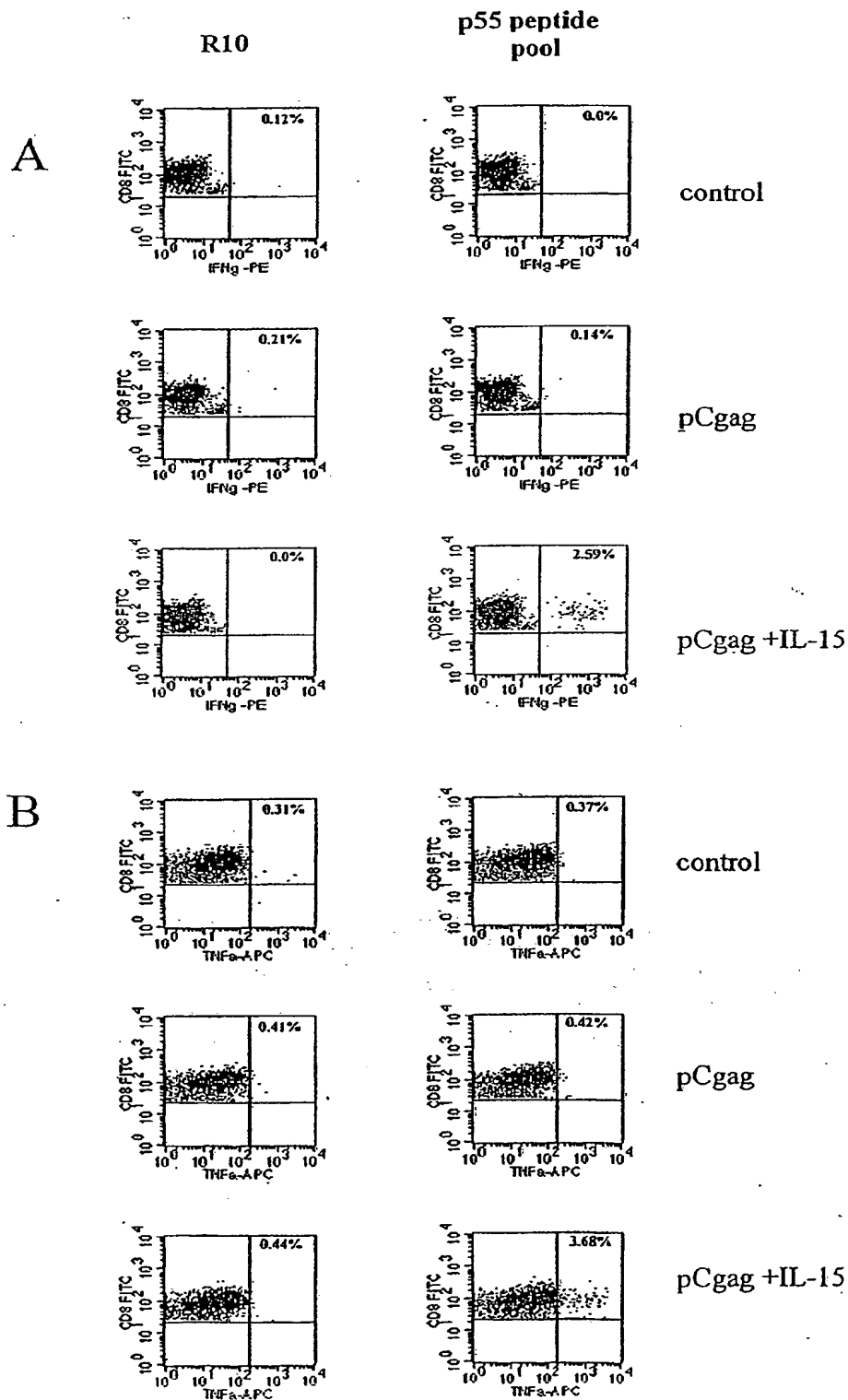


Figure 5

BEST AVAILABLE COPY

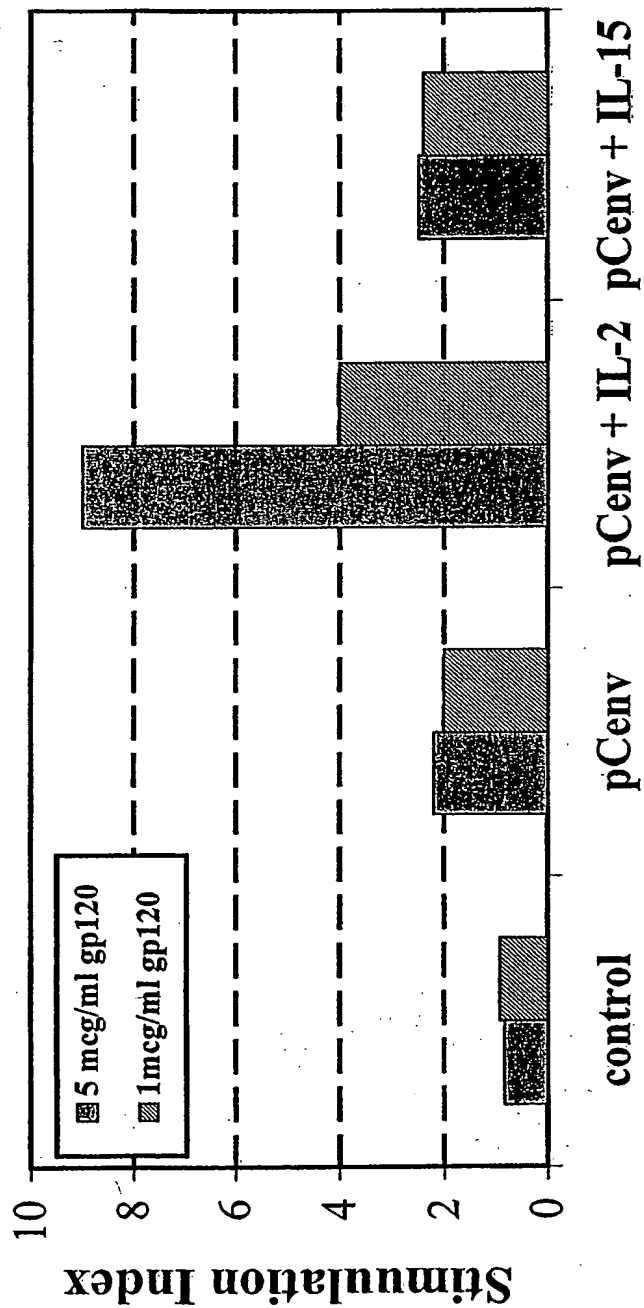


FIGURE 6

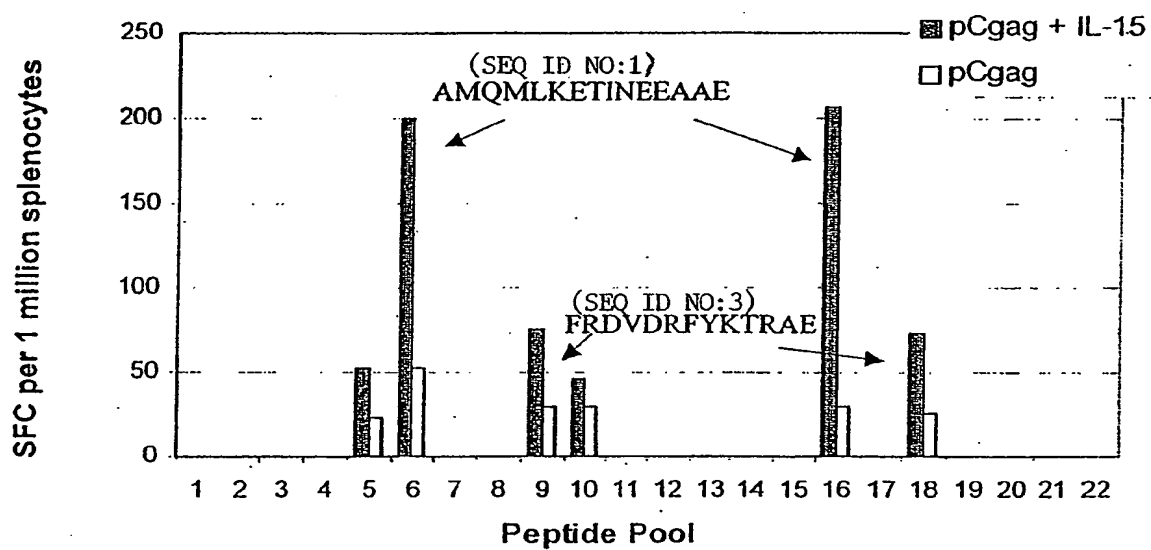


Figure 7

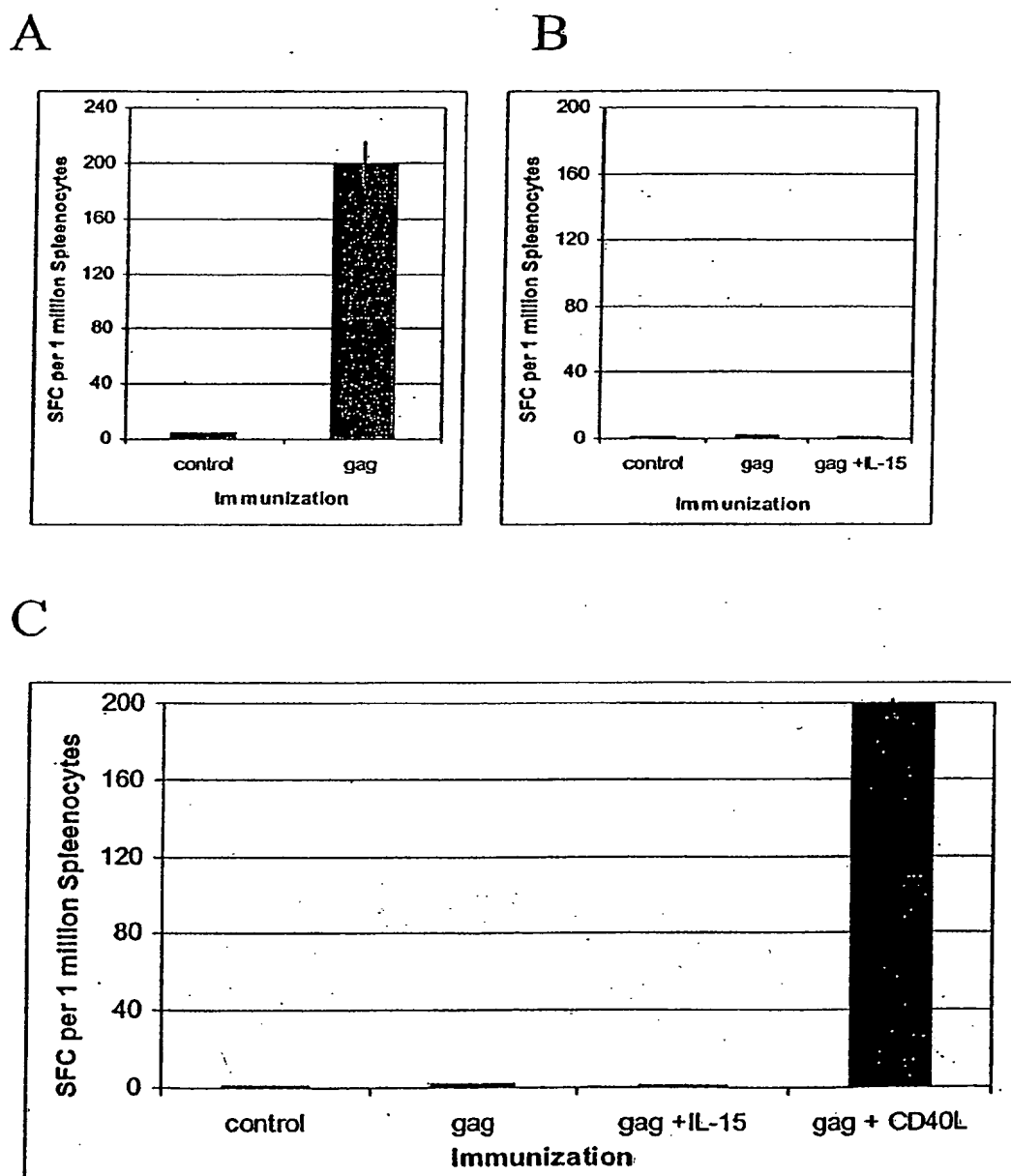


Figure 8

11/18

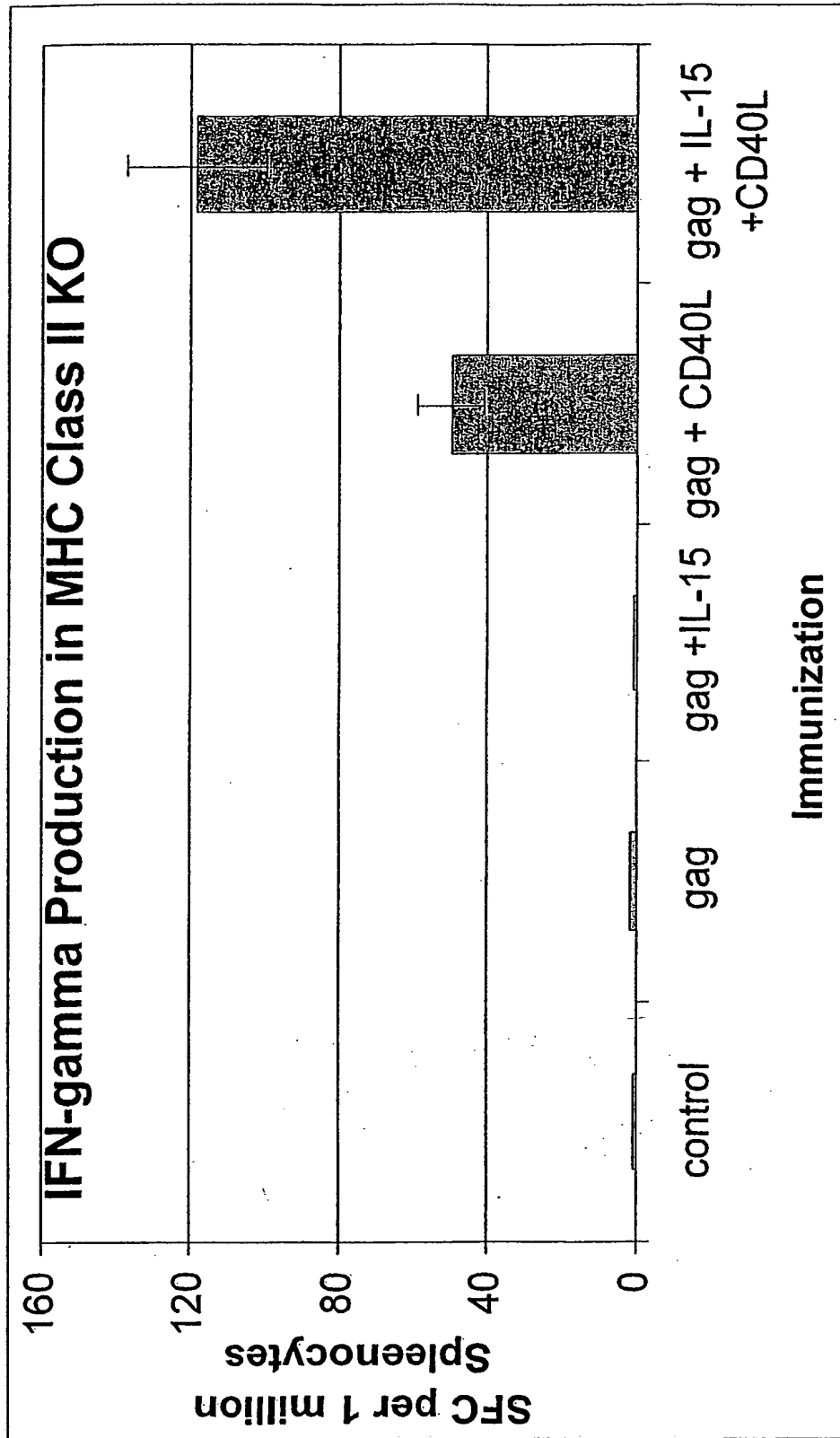


FIGURE 9

# Strategy for Increasing Expression of IL-15 through Optimization of IL-15 DNA Constructs for Immunization

- ❖ Primers are designed to amplify IL-15 from start of signal peptide, thus upstream inhibitory AUGs are not present in the final IL-15 message.
- ❖ Primers are designed to include a strong KOZAK context (GCCGCCACC).
- ❖ Removal of the C-terminus negative regulatory element using PCR antisense primer design

Primer Name	Sense/Antisense	Sequence 5' to 3'
Human IL-15 (LSP)	sense (SEQ ID NO:4)	GCGCCCGTCGAC GCCGCCACC ATGAGAAATTCGAAACCACATTGAG
	antisense (SEQ ID NO:5)	ATCGGGCTCGAG TCAAGAAGTGTGATGAACATTGG
Macaque IL-15 (LSP)	sense (SEQ ID NO:4)	GCGCCCGTCGAC GCCGCCACC ATGAGAAATTCGAAACCACATTGAG
	antisense (SEQ ID NO:5)	ATCGGGCTCGAG TCAAGAAGTGTGATGAACATTGG
Human IL-15 (SSP)	sense (SEQ ID NO:6)	GCGCCCGGTACC GCCGCCACC ATGGTATTGGGAACCATA
	antisense (SEQ ID NO:7)	ATCGGGGGATCC TCAAGAAGTGTGATGAACAT
Legend: Restriction Site, KOZAK, START, STOP CODON		

FIGURE 10

BEST AVAILABLE COPY

## Strategy for Increasing Expression of IL-15 through Replacement of 48 amino acid Signal Peptide (LSP) with IgE leader

- ❖ Sense primers are designed to start after 48 aa ISP while antisense primer amplifies from stop site.
- ❖ Primers are designed to include a strong KOZAK context (GCCGCCACC).
- ❖ Sense primer is designed to contain the sequence for IgE leader sequence plus a ATG start site.

Primer Name	Sense/Antisense	Sequence 5' to 3'
Human IL-15-IgE	sense (SEQ ID NO: 8)	GCCCGCGAAATTC GCCGCCACCATGGATTGGACTTGGATCTTATTTT
	(SEQ ID NO: 9)	AGTTGCTGCTGCTACTAGAGTTCAATCTAACTGGGTGAATGTAATAAGT
	antisense (SEQ ID NO: 5)	ATCGGGCTCGAG TCAAGAAGTGTGATGAACATTGG
	sense (SEQ ID NO: 8)	GCCCGCGAAATTC GCCGCCACCATGGATTGGACTTGGATCTTATTTT
Macaque IL-15-IgE	(SEQ ID NO: 9)	AGTTGCTGCTGCTACTAGAGTTCAATCTAACTGGGTGAATGTAATAAGT
	antisense (SEQ ID NO: 5)	ATCGGGCTCGAG TCAAGAAGTGTGATGAACATTGG
	sense (SEQ ID NO: 8)	GCCCGCGAAATTC GCCGCCACCATGGATTGGACTTGGATCTTATTTT
	(SEQ ID NO: 9)	AGTTGCTGCTGCTACTAGAGTTCAATCTAACTGGGTGAATGTAATAAGT

Legend: Restriction Site, KOZAK, START, STOP CODON

FIGURE 11

BEST AVAILABLE COPY

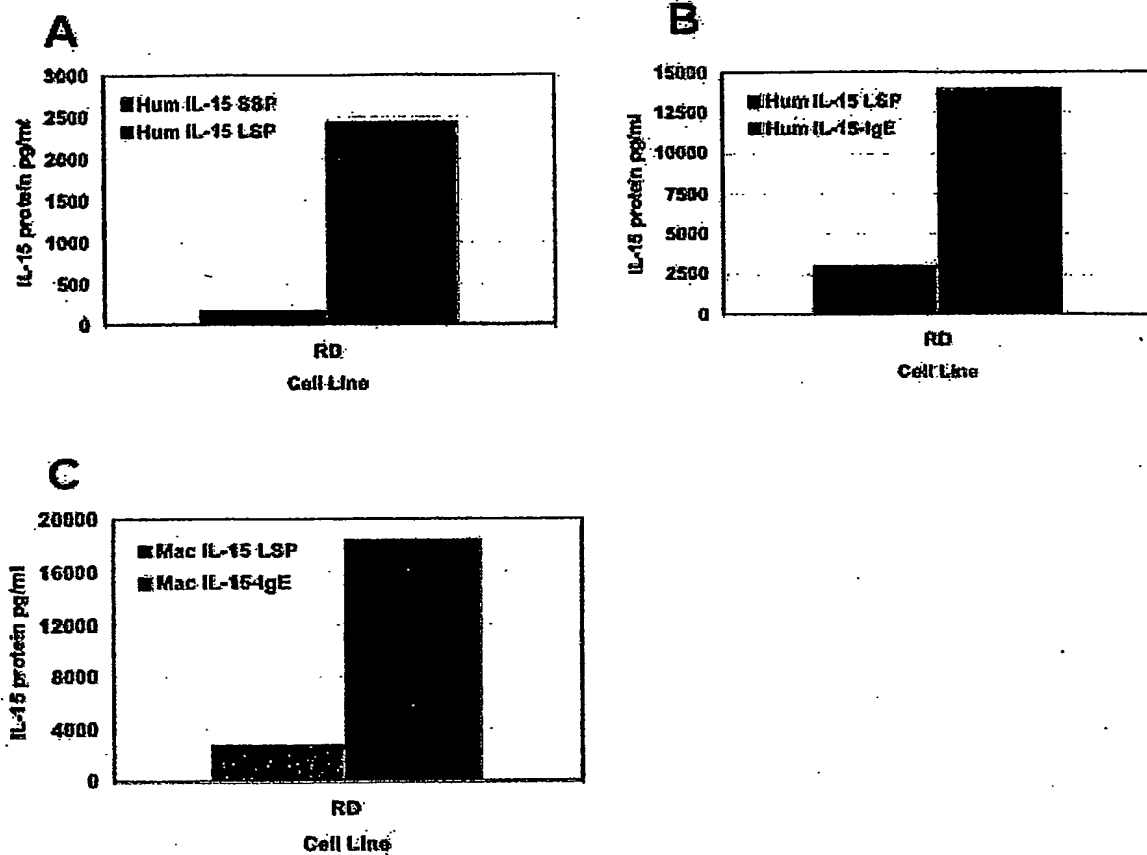


FIGURE 12

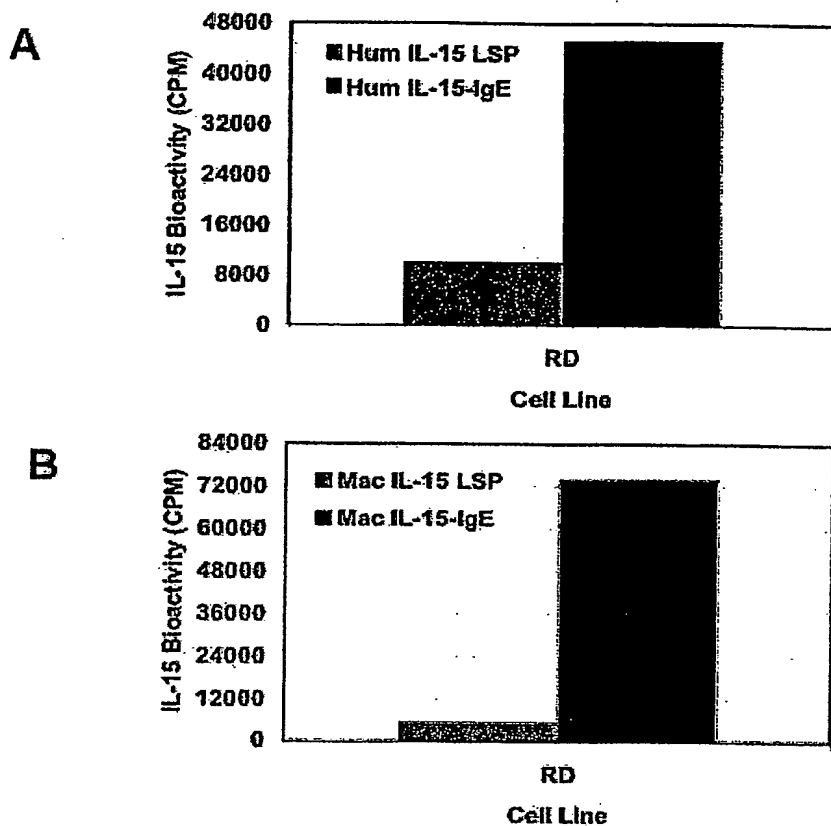


FIGURE 13

# Immunization Schedule

Immunization Groups:

Naïve

Vector Control

HIV-1 Gag

HIV-1 Gag/ IL15 constructs



*Combinations of 100 µg IL15 Constructs, 50 µg GAG,  
Each injection, intramuscular*

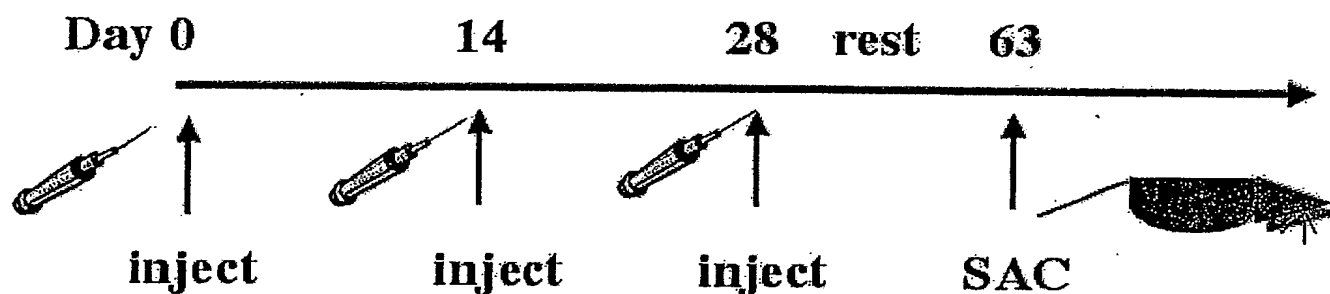


FIGURE 14

BEST AVAILABLE COPY

Restimulation of antigen-specific IFN- $\gamma$  production 5 Weeks Following  
the 3rd immunization of HIV-1 Gag in Balb/C mice  
*Effect of IL-15 Constructs*

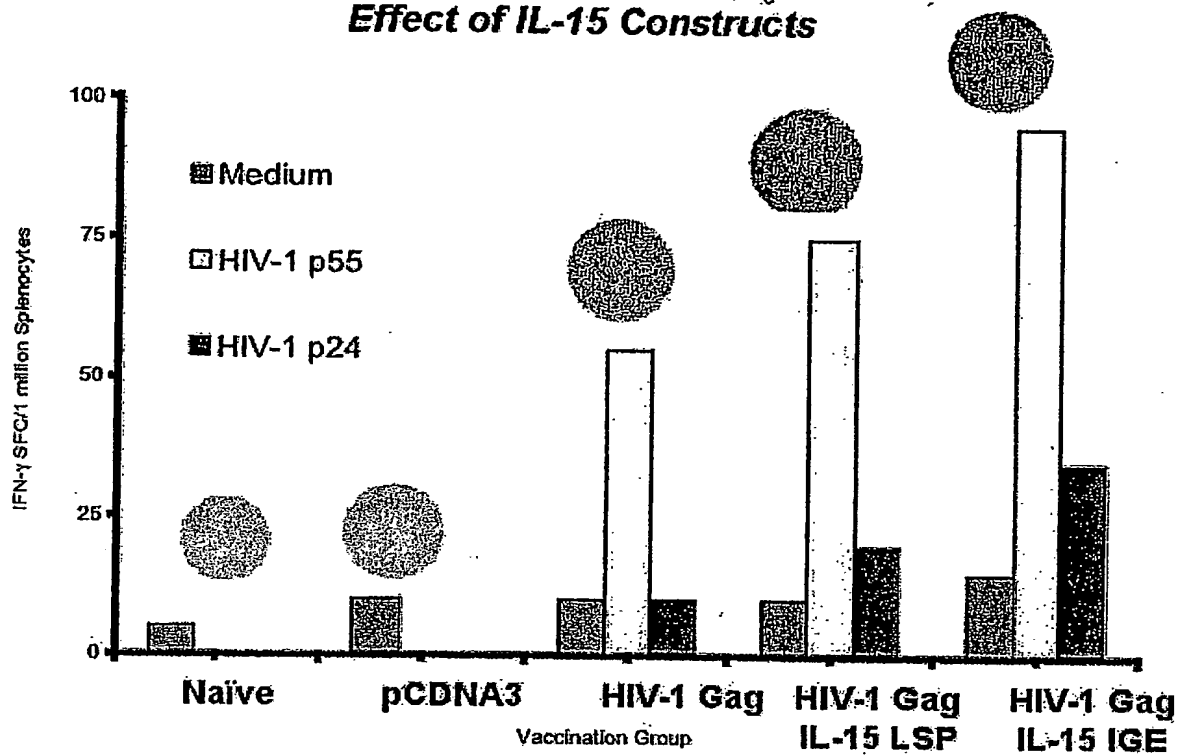
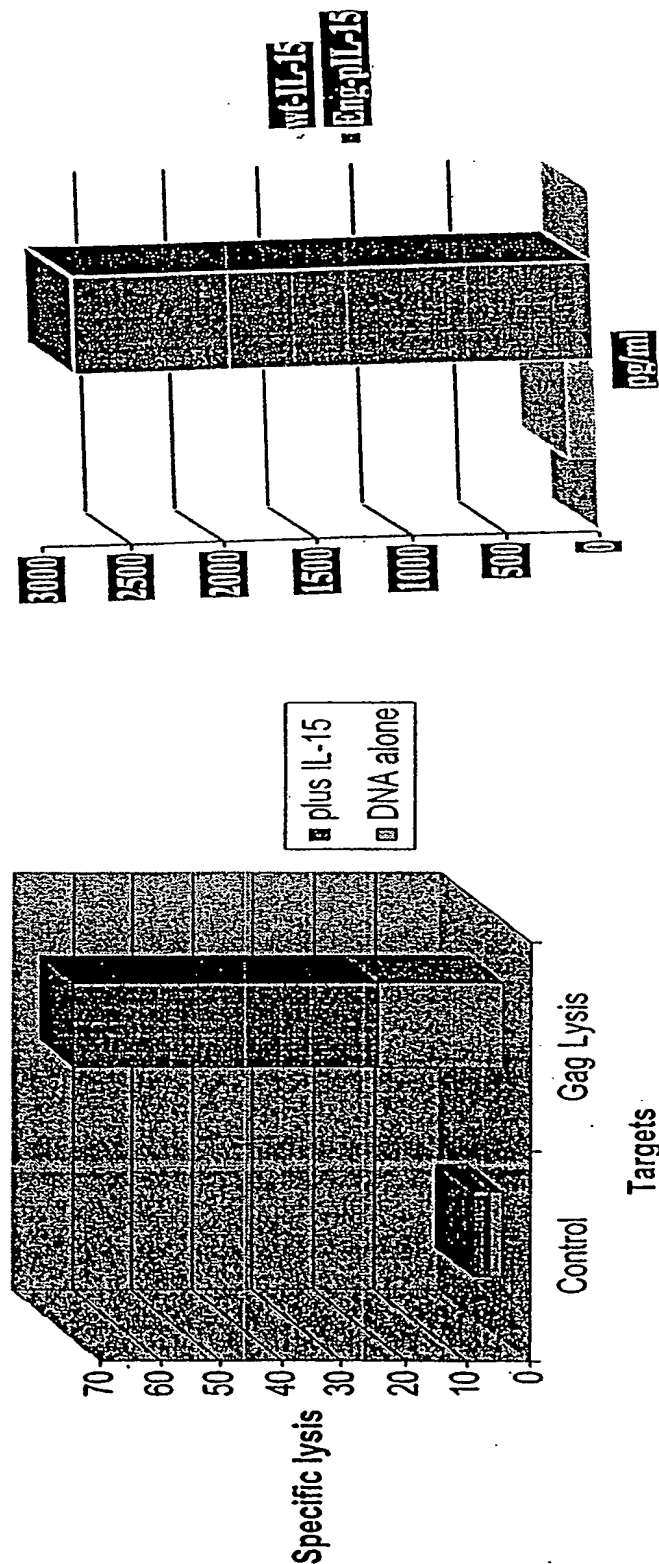


FIGURE 15

# An Engineered IL-15 Plasmid Vaccine (Kozak, AUG's removed, UTR's removed & other-30- 50X better expression)

Enhances CTL response in Vivo

Mice were immunized with HIV-1 gag expressing DNA



Grabstein et al. (1994) Science 264:965-968, Bamford et al., 1996) PNAS 93:2897-2902  
Bamford et al., (1998) J. Immunol 160:4418-4426, Kozak et al., (1991) J. Cell Biol. 115:887-903

FIGURE 16

BEST AVAILABLE COPY